JOURNAL OF THE
WORLD AQUACULTURE SOCIETY

Vol. 40, No. 1
February, 2009

Impact of Copper Sulfate on Plankton in Channel Catfish Nursery Ponds

Charles C. Mischke[1] and David J. Wise

Thad Cochran National Warmwater Aquaculture Center, Mississippi State University, PO Box 197, Stoneville, MS 38776 USA

Paul V. Zimba

United States Department of Agriculture, Agricultural Research Service, Catfish Genetics Research Unit, PO Box 38, Stoneville, MS 38776 USA

Abstract

Many fish culturists are interested in applying copper sulfate pentahydrate (CSP) to channel catfish, *Ictalurus punctatus*, nursery ponds as a prophylactic treatment for trematode infection and proliferative gill disease by killing snails and *Dero* sp., respectively, before stocking fry. However, copper is an algaecide and may adversely affect phytoplankton and zooplankton populations. We evaluated the effects of prophylactic use of copper sulfate in catfish nursery ponds on water quality and phytoplankton and zooplankton populations. In 2006, treatments of 0 mg/L CSP, 3 mg/L CSP (0.77 mg/L Cu), and 6 mg/L CSP (1.54 mg/L Cu) were randomly assigned to 0.04-ha ponds. In 2007, only treatments of 0 and 3 mg/L CSP were randomly assigned to the 16 ponds. Ponds treated with CSP had significantly higher pH and significantly lower total ammonia concentrations. Treatment of both CSP rates increased total algal concentrations but reduced desirable zooplankton groups for catfish culture. CSP has been shown to be effective in reducing snail populations at the rate used in this study. CSP treatment also appears to be beneficial to the algal bloom, shifting the algal population to green algae and increasing total algal biomass within 1 wk after CSP treatment. Although zooplankton populations were adversely affected, populations of important zooplankton to catfish fry began rebounding 6–12 d after CSP treatment. Therefore, if CSP is used to treat catfish fry ponds of similar water composition used in this study, fry should not be stocked for about 2 wk after CSP application to allow time for the desirable zooplankton densities to begin increasing.

Copper products have been used in aquaculture as algaecides for many years. Although copper is not considered to be selective, it has been used successfully to control the specific algae that cause off-flavors in catfish ponds (Tucker et al. 2001; Schrader et al. 2005). Application of copper sulfate pentahydrate (CSP) can also be effective in controlling snails in channel catfish, *Ictalurus punctatus*, production ponds. Snails, specifically the marsh rams-horn snail, *Planorbella trivolvis*, serve as intermediate hosts of the digenetic trematode *Bolbophorus damnificus*. This trematode has caused millions of dollars in economic losses and especially impacts fingerling catfish (Mitchell 2001). Reduction of snail populations is the most practical method of controlling this trematode. Copper is toxic to snails, and in laboratory studies, the 24-h LC_{50} of copper was 0.6 mg/L (Mischke et al. 2005). Shoreline treatments with CSP are effective in killing snails along the pond margins (Mitchell 2002; Mitchell and Hobbs 2003) as is uniform application of CSP throughout the entire pond at a rate of 2.5–5.0 mg/L (0.64–1.27 mg/L Cu) (Wise et al. 2006).

In addition to snail control, copper may be useful in reducing *Dero* spp. populations. The oligochaete *Dero digitata* is an intermediate host to the parasite that causes proliferative gill disease in channel catfish. As with trematodes, reduction of the intermediate host is the most practical method of controlling the disease. In laboratory studies, *Dero* spp. appeared to be sensitive to copper exposure with the LC_{50} values decreasing from 7.6 mg/L at 24 h to 1.8 mg/L at 48 h (Mischke et al. 2001).

[1] Corresponding author.

Because CSP application controls snail populations and has the potential to reduce *Dero* spp. populations, many fish culturists are interested in applying CSP to channel catfish nursery ponds as a prophylactic treatment before stocking fry. However, copper is an algaecide and may adversely affect phytoplankton and zooplankton. Tolerance of algae to copper varies widely among algal groups, and LC_{50} values from 0.004 mg/L Cu for certain Cyanophyceae to 3.132 mg/L Cu for certain Chlorophyceae have been reported (Takamura et al. 1990). Zooplankton appear to be very sensitive to copper. The LC_{50} of 44 freshwater cladocerans ranged from 0.005 to 0.071 mg/L Cu (Bossuyt and Janssen 2005).

The purpose of this study was to evaluate the effects of prophylactic use of CSP in catfish nursery ponds on phytoplankton and zooplankton populations and water quality variables.

Materials and Methods

Studies were conducted in 16 earthen ponds (0.04 ha) constructed on alluvial clay soils of the Yazoo–Mississippi River floodplain at the Thad Cochran National Warmwater Aquaculture Center, Stoneville, Mississippi. Ponds were filled with well water supplied from the Mississippi River alluvial aquifer in mid-April. Fertilization of all ponds began immediately, following the protocol for catfish nursery ponds (Mischke and Zimba 2004). Fertilization continued throughout the entire study both years.

In 2006, treatments of 0 mg/L CSP (control; $n = 4$), 3 mg/L CSP (0.77 mg/L Cu; $n = 6$), and 6 mg/L CSP (1.54 mg/L Cu; $n = 6$) were randomly assigned. In 2007, only treatments of 0 and 3 mg/L CSP were randomly assigned to the 16 ponds. The required amount of CSP for each pond was dissolved in water and broadcast over the pond surface.

In both studies, water quality sampling occurred weekly. In 2006, daily samples were analyzed for phytoplankton and zooplankton; in 2007, twice-weekly samples were collected throughout the study. Water samples were collected by taking five tube samples (Graves and Morrow 1998) from the northeastern and southwestern corners of each pond (water depth = 1.3 m) and combined to obtain a total water volume of about 8 L. Chemical and biological variables from each pond were estimated from subsamples of this composite sample.

Ammonia (nesslerization), nitrite (diazotization), nitrate (cadmium reduction followed by diazotization), total phosphorus (persulfate oxidation/digestion and ascorbic acid finish), soluble reactive phosphorus (ascorbic acid method), and total nitrogen (persulfate oxidation) were determined using methods outlined by HACH (1999).

Pigment analysis was used to assess phytoplankton community composition by using high performance liquid chromatography (HPLC) methodology (Zimba et al. 1999). Pigments (carotenoids and chlorophylls) were quantified with an HP1100 equipped with diode array and fluorescence detectors (Agilent Technologies, Palo Alto, CA, USA). Identification of specific divisions of algae is possible by using taxon-specific pigment biomarkers (Zimba et al. 2002). A pigment library was used to identify samples; unknown samples were quantified by linear regression of known commercial standards.

Zooplankton samples were collected with oblique 2-m tows with a 63-μm mesh Wisconsin-style net (Wildlife Supply, Saginaw, MI, USA) from each pond. A mark was placed on the net's towrope at 2 m, and a total of 23 L of pond water were filtered for each zooplankton sample. Samples were concentrated and preserved in 240 mL of buffered formalin solution before counting by light microscopy (Geiger and Turner 1990). All organisms in 1-mL subsamples from each pond were counted using a Sedgwick–Rafter counting cell as described by Geiger and Turner (1990) and identified with the taxonomic keys of Thorp and Covich (1991).

For both studies, the analysis was for a completely randomized design with repeated measures taken on ponds. Data were analyzed with the MIXED procedure in SAS Version 8.02 software (SAS Institute, Inc., Cary, NC, USA) (Littell et al. 1996). The covariance structure, autoregressive of order 1, was used in the repeated measure model. Mean comparisons were made using an LSD test with a significance level of $P \leq 0.05$.

TABLE 1. *Least square means of water quality variables (mg/L) in ponds treated with 0, 3, and 6 mg/L copper sulfate pentahydrate (CSP) (2006) and 0 and 3 mg/L CSP (2007).*

2006				
Treatment	NO_3	NH_3	NO_2	pH
0 mg/L CSP	0.30	0.826	0.028	8.0
3 mg/L CSP	0.27	0.724	0.0171	8.4
6 mg/L CSP	0.29	0.636	0.0169	8.3
ANOVA: *P* values				
Treatment	0.8588	0.2105	0.0843	0.0029
Date	0.0001	0.0001	0.0001	0.0001
Interaction	0.7819	0.0655	0.0001	0.0001
2007				
Treatment	NO_3	NH_3	NO_2	pH
0 parts	—	1.488	—	7.9
3 parts	—	1.014	—	8.0
ANOVA: *P* values				
Treatment	—	0.0165	—	0.0346
Date	—	0.0001	—	0.0001
Interaction	—	0.0795	—	0.0026

Results

Total hardness and total alkalinity ranged from 150 to 200 mg/L $CaCO_3$. Water temperature at the time of copper treatment was 23 C in 2006 and 20 C in 2007. Temperatures were warmer during the study in 2006 (20–25 C) than in 2007 (12–21 C). Water quality variables were mostly unaffected by copper treatment; however, pH in copper-treated ponds was slightly but significantly higher ($P < 0.05$) in both years (Table 1). Also, in 2007, total ammonia nitrogen concentrations were significantly reduced in CSP-treated ponds.

CSP increased total phytoplankton biomass after treatment (Table 2). Chlorophyll *a* (total algal biomass indicator) increased with increasing CSP treatment rate (Fig. 1). Phaeophytin (an indicator of algal breakdown/senescence) increased as CSP rate increased, paralleling chlorophyll measurements. Lutein (a biomarker for chlorophytes) increased at the lowest CSP concentration but declined at the highest CSP concentration. Fucoxanthin, a biomarker for diatoms, decreased in 2006 by threefold, yet was unaffected by CSP treatment in 2007.

As expected, CSP treatment significantly impacted zooplankton populations (Table 3). Rotifer numbers were reduced initially after CSP treatment but were higher than control ponds by the end of the studies. For catfish fry, copepods, cladocerans, and ostracods are the important zooplankton forage taxa (Mischke et al. 2003). This combined group of zooplankton was impacted by CSP treatment. Population densities were significantly reduced immediately after CSP treatment and required 6–12 d

TABLE 2. *Least square means of phytopigment concentrations (μg/L) in ponds treated with 0, 3, and 6 parts copper sulfate pentahydrate (CSP) (2006) and 0 and 3 parts CSP (2007).*

2006									
Treatment	Phaeo	C2	Fucox	Neox	Violax	Diadino	Lutein	Chlorophyll *b*	Chlorophyll *a*
0 mg/L CSP	0.320	0.984	0.256	0.052	0.064	0.126	0.225	0.274	2.565
3 mg/L CSP	2.880	0.572	0.150	0.274	0.257	0.174	1.057	1.038	5.934
6 mg/L CSP	5.834	0.551	0.086	0.399	0.403	0.170	0.121	1.124	7.000
ANOVA: *P* values									
Treatment	0.0151	0.3962	0.0862	0.0001	0.0001	0.7730	0.0002	0.0004	0.0005
Date	0.0001	0.0008	0.0001	0.0001	0.0001	0.0637	0.0001	0.0001	0.0001
Interaction	0.0001	0.5318	0.8638	0.0001	0.0001	0.9926	0.0001	0.0001	0.0001
2007									
Treatment	Phaeo	C2	Fucox	Neox	Violax	Diadino	Lutein	Chlorophyll *b*	Chlorophyll *a*
0 mg/L CSP	0.103	0.262	0.076	0.044	0.049	0.033	0.184	0.217	1.926
3 mg/L CSP	0.653	0.251	0.0597	0.232	0.265	0.085	0.954	1.253	7.153
ANOVA: *P* values									
Treatment	0.0086	0.8934	0.3510	0.0001	0.0001	0.0103	0.0001	0.0001	0.0001
Date	0.0041	0.0012	0.0001	0.0001	0.0001	0.0049	0.0001	0.0001	0.0001
Interaction	0.0268	0.0274	0.4526	0.0001	0.0001	0.0040	0.0001	0.0001	0.0001

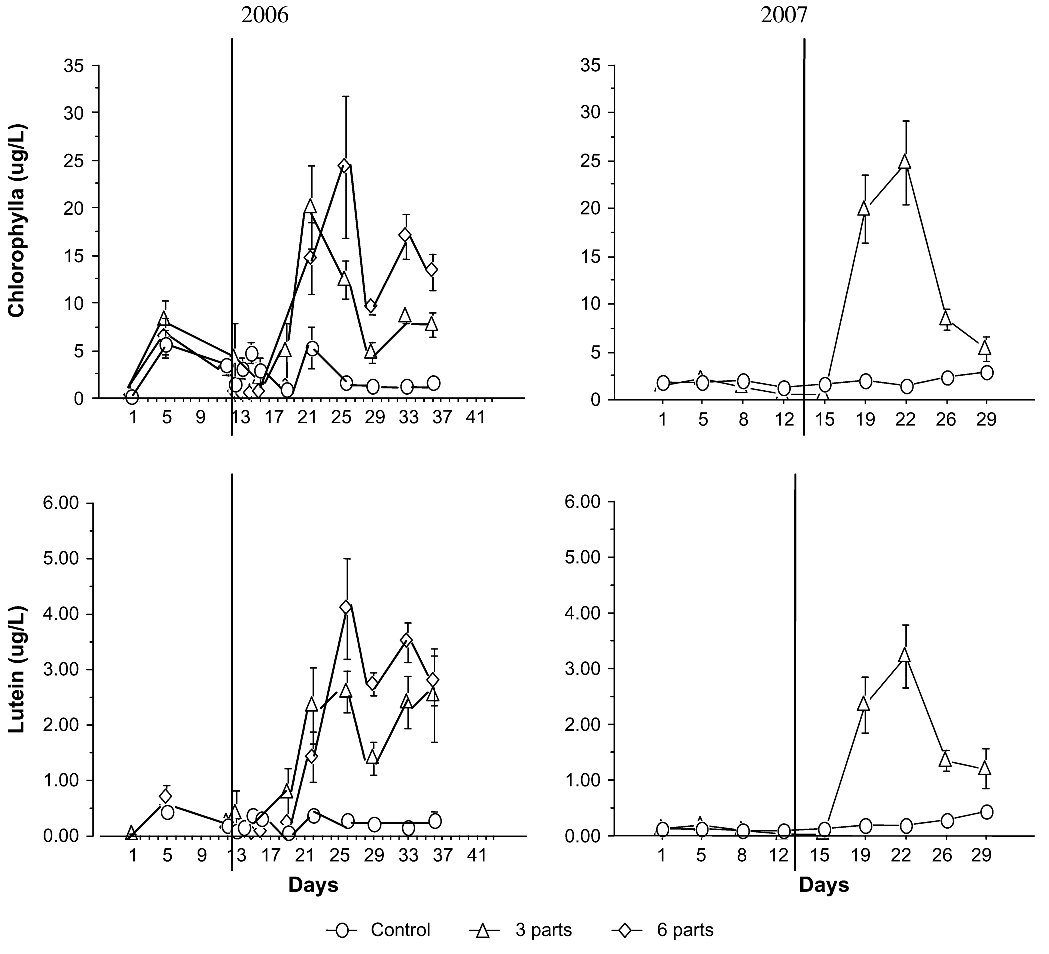

FIGURE 1. *Mean (±SE) concentrations of chlorophyll* a *and lutein in ponds treated with 0, 3, and 6 mg/L copper sulfate pentahydrate (CSP) (2006) and 0 and 3 mg/L CSP (2007). The vertical bar indicates the date of CSP application.*

before densities began increasing again (Fig. 2).

Discussion

Effects of either CSP application rate on water quality were minimal, but there did tend to be reduced total ammonia concentrations in CSP-treated ponds. Although total ammonia concentrations were reduced in treated ponds, unionized ammonia concentrations were higher because of the concurrent increase in pH. The increase in pH and decrease in ammonia in CSP-treated ponds are probably related to the increase in overall algal biomass.

Increases in algal biomass are common after sublethal copper exposure (Tripathi and Gaur 2006; Wilde et al. 2006; Perales-Vela et al. 2007) as well as sublethal exposure to other herbicides (Kowano et al. 2005). In these studies, samples were collected on the scale of hours–days, whereas we collected samples on the timescale of days–weeks. Significantly higher chlorophyll *a* and total carotenoid cellular concentrations were reported by Perales-Vela et al. (2007) for *Scenedesmus* sp. exposed to micromolar Cu concentrations. Cid et al. (1995) reported that 1 mg/L free copper inhibited growth in the marine diatom *Phaeodactylum tricornutum* while photosynthetic rates were less impacted.

Based on the measured increases in chlorophyll *a* and lutein, it appears that copper treatments

TABLE 3. *Least square means of zooplankton (number/L) in ponds treated with 0, 3, and 6 mg/L copper sulfate pentahydrate (CSP) (2006) and 0 and 3 mg/L CSP (2007).*

Treatment	Rotifers	Copepods	Cladocerans	Nauplii	Desirable zooplankton
2006					
0 mg/L CSP	803	140	53	401	207
3 mg/L CSP	640	64	10	148	80
6 mg/L CSP	883	34	11	109	52
ANOVA: *P* values					
Treatment	0.5261	0.0007	0.0009	0.0009	0.0002
Date	0.0001	0.0001	0.0001	0.0001	0.0001
Interaction	0.0001	0.0001	0.0403	0.0083	0.0001
2007					
Treatment	Rotifers	Copepods	Cladocerans	Nauplii	Desirable zooplankton
0 parts	322	62	31	271	97
3 parts	409	47	9	121	58
ANOVA: *P* values					
Treatment	0.5995	0.2972	0.0001	0.0272	0.01500
Date	0.0043	0.0004	0.0191	0.0001	0.0002
Interaction	0.3772	0.0143	0.0001	0.3288	0.0045

altered the phytoplankton composition, increasing the concentration of green algae. This result is consistent based on lethal concentrations reported by Takamura et al. (1990). All Cyanophyceae tested had Cu EC50 values <0.390 mg/L, but Chlorophyceae had Cu EC50 values as high as 3.132 mg/L. Numerous studies have shown different mortalities of algae as a function of copper form, pH, temperature, and copper concentration. As copper uptake by the algae is controlled at the cell surface by competitive binding (Webster and Gadd 1996; Tripathi and Gaur 2006; Wilde et al. 2006; Perales-Vela et al. 2007), differences within the same genus or even species in terms of response to a specific copper dosage are understandable. Diatoms appeared to be only slightly impacted by CSP treatment.

The drastic decrease in zooplankton population densities was expected based on the reported toxicity of copper for cladocerans of

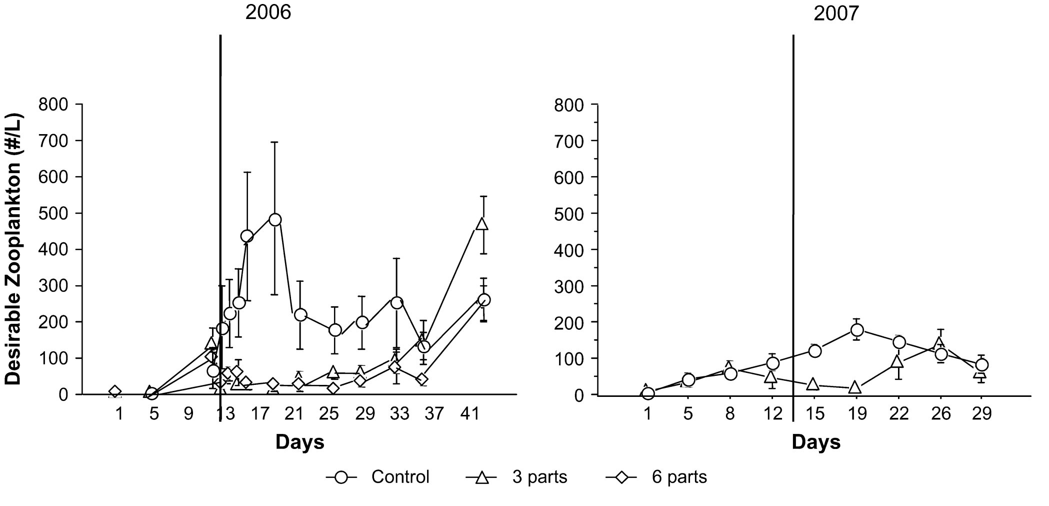

FIGURE 2. *Mean (±SE) concentrations of desirable zooplankton (total of copepods, cladocerans, and ostracods) in ponds treated with 0, 3, and 6 mg/L copper sulfate pentahydrate (CSP) (2006) and 0 and 3 mg/L CSP (2007). The vertical bar indicates the date of CSP application.*

<0.071 mg/L Cu compared to our treatment levels of 0.77 and 1.54 mg/L Cu (Bossuyt and Janssen 2005). Zooplankton populations appeared to need 6–12 d to begin increasing in density after CSP treatment.

Prophylactic treatment of catfish nursery ponds with CSP may be beneficial. CSP is effective in reducing snail populations at the rate used in this study (Wise et al. 2006). CSP treatments at the rates used in this study also appear to be beneficial to the algal bloom, shifting the algal population to green algae and increasing total algal biomass within 1 wk after CSP treatment. Although zooplankton populations were adversely affected, populations of important zooplankton to catfish fry began rebounding 6–12 d after CSP treatment. Therefore, if CSP is used to treat catfish fry ponds of similar water composition used in this study, fry should not be stocked for about 2 wk after CSP application to allow time for the desirable zooplankton densities to begin increasing. Although copper levels were not measured in this study, by 2–3 wk postapplication, copper is probably removed from solution through biological processes and adsorption (Boyd 1990) and should be at safe levels for stocking fry. Waiting for this amount of time before stocking fry could allow time for predacious insects to become established in the ponds, and appropriate control methods would be required.

Acknowledgments

This is journal article no. J-11200 of the Mississippi Agricultural and Forestry Experiment Station (MAFES), Mississippi State University. This project is supported under MAFES Project Number MIS-0811.

Literature Cited

Bossuyt, B. T. A. and C. R. Janssen. 2005. Copper toxicity to different field-collected cladoceran species: intra- and inter-species sensitivity. Environmental Pollution 136:145–154.

Boyd, C. E. 1990. Water quality in ponds for aquaculture. Alabama Agricultural Experiment Station, Auburn University, Auburn, Alabama, USA.

Cid, A., C. Herrero, E. Torres, and J. Abalde. 1995. Copper toxicity in the marine microalgae *Phaeodactylum tricornutum*: effects on photosynthesis and related parameters. Aquatic Toxicology 31:165–174.

Geiger, J. G. and C. J. Turner. 1990. Pond fertilization and zooplankton management techniques for production of fingerling striped bass and hybrid striped bass. Pages 79–98 *in* R. M. Harrell, J. H. Kerby, and R. V. Minton, editors. Culture and propagation of striped bass and its hybrids. American Fisheries Society, Bethesda, Maryland, USA.

Graves, K. G. and J. C. Morrow. 1998. Tube sampler for zooplankton. Progressive Fish-Culturist 50:182–183.

HACH. 1999. DR/4000 spectrophotometer procedure manual. HACH Chemical, Loveland, Colorado, USA.

Kowano, T., T. Kosaka, and H. Aosaya. 2005. Impact of sulfonylureic herbicide on growth of photosynthetic and non-photosynthetic protozoa. Pages 495–504 *in* E. Lichtfouse, J. Schwarzbauer, and D. Robert, editors. Environmental Chemistry. Springer, Berlin, Germany.

Littell, R. C., G. A. Milliken, W. W. Stroup, and R. D. Wolfinger. 1996. SAS system for mixed models. SAS Institute, Inc., Cary, North Carolina, USA.

Mischke, C. C. and P. V. Zimba. 2004. Plankton community responses in earthen channel catfish nursery ponds under various fertilization regimes. Aquaculture 233:219–235.

Mischke, C., J. Terhune, and D. Wise. 2001. Acute toxicity of several chemicals to the oligochaete *Dero digitata*. Journal of the World Aquaculture Society 32:184–188.

Mischke, C. C., D. J. Wise, and R. L. Lane. 2003. Zooplankton size and taxonomic selectivity of channel catfish fry. North American Journal of Aquaculture 65:141–146.

Mischke, C. C., D. J. Wise, and L. M. Pote. 2005. Acute toxicity of chemicals to the marsh rams-horn snail *Planorbella trivolvis*. Journal of the World Aquaculture Society 36:560–563.

Mitchell, A. J. 2001. Update and impact of a trematode that infects cultured channel catfish. The Catfish Journal 16:17–27.

Mitchell, A. J. 2002. A copper sulfate-citric acid pond shoreline treatment to control the rams-horn snail *Planorbella trivolvis*. North American Journal of Aquaculture 64:182–187.

Mitchell, A. J. and M. S. Hobbs. 2003. Effect of citric acid, copper sulfate concentration, and temperature on a pond shoreline treatment for control of the marsh ram's horn snail *Planorbella trivolvis* and the potential toxicity of the treatment to channel catfish. North American Journal of Aquaculture 65:306–313.

Perales-Vela, H. V., S. González-Moreno, C. Montes-Horcasitas, and R. O. Cañizares-Villanueva. 2007. Growth, photosynthetic, and respiratory responses to sublethal copper concentrations in *Scenedesmus incrassatulus* (Chlorophyceae). Chemosphere 67:2274–2281.

Schrader, K. K., C. S. Tucker, T. R. Hanson, P. D. Gerard, S. K. Kingsbury, and A. M. Rimando. 2005. Management of musty off-flavor in channel catfish from commercial ponds with weekly applications of copper sulfate. North American Journal of Aquaculture 67:138–147.

Takamura, N., F. Kasai, and M. M. Watanabe. 1990. Unique response of Cyanophyceae to copper. Journal of Applied Phycology 2:293–296.

Thorp, J. H. and A. P. Covich. 1991. Ecology and classification of North American freshwater invertebrates. Academic Press, San Diego, California, USA.

Tripathi, B. N. and J. P. Gaur. 2006. Physiological behavior of *Scenedesmus* sp. during exposure to elevated levels of Cu and Zn after withdrawal of stress. Protoplasma 229:1–9.

Tucker, C. S., T. R. Hanson, and S. K. Kingsbury. 2001. Management of off-flavors in pond-cultured channel catfish with weekly applications of copper sulfate. North American Journal of Aquaculture 63:118–130.

Webster, E. A. and G. M. Gadd. 1996. Perturbation of monovalent cation composition in *Ulva lactuca* by cadmium, copper and zinc. Biometals 9:51–56.

Wilde, K. L., J. L. Stauber, S. J. Markich, N. M. Franklin, and P. L. Brown. 2006. The effect of pH on the uptake and toxicity of copper and zinc in a tropical freshwater algae (*Chlorella* sp.). Archives of Environmental Contamination Toxicology 51:174–185.

Wise, D. J., C. C. Mischke, T. Greenway, T. S. Byars, and A. J. Mitchell. 2006. Uniform application of copper sulfate as a potential treatment for controlling snail populations in channel catfish production ponds. North American Journal of Aquaculture 68: 364–368.

Zimba, P. V., C. P. Dionigi, and D. F. Millie. 1999. Evaluating the relationship between photopigment synthesis and 2-methylisoborneol accumulation in cyanobacteria. Journal of Phycology 35:1422–1429.

Zimba, P. V., C. S. Tucker, C. C. Mischke, and C. C. Grimm. 2002. Short-term effect of diuron on catfish pond ecology. North American Journal of Aquaculture 64:16–23.